to distinguish from immature specimens of the present variety of *pseudo-monticola*. Only the collection of complete specimens (flowers, fruit, and mature leaves from fruiting branchlets, and mature seasonal shoots) will enable the final solution of this problem.

The widespread and discriminating observations and collecting of Professor Edward C. Smith of the Department of Botany, Colorado Agricultural College, is producing information and material of much value in the solution of this and other problems of the Colorado willows. He recently has raised the question of the taxonomic status of the representatives of the *Commutatae* in that State and this contribution is a partial answer to the question. More complete collecting may make a complete answer possible.

It is probable that some of the mature-foliage specimens now assigned to *S. pseudo-monticola* really belong to the variety but this can be determined only after flowering, fruiting, and late foliage specimens from the same plant are available for study. The following flowering and fruiting specimens are referred to this variety.

Specimens examined

ARIZONA (east central): White Mts., south of Thomas Peak, Coville 2009 (USN), July 2, 1904.

COLORADO (western): *Gunnison Co.*, Vicinity of Mt. Carbon, elev. 2750 m., Eggleston 5671 (USN), June 11, 1910. *Hinsdale Co.* (Gunnison Watershed), Carson, elev. 11,000 ft., Baker 306 (USN), July 2, 1901. *Monterey* or *La Plata Co.*, Bob Creek, West La Plata Mts., elev. 10,000 ft., Baker, Earle, and Tracy 175 (USN, cited by Rydberg), June 28, 1898.

COLORADO (eastern): *Chaffee Co.*, Buena Vista, Eastwood 7071 (USN), June 18, 1918. *Conejos Co.*, Los Pinos, elev. 7000 ft., Baker 271 (USN, cited by Rydberg), May, 1899. *El Paso Co.*, Zanger Farm, Black Squirrell Creek, Christ 1955 (CRB), May 5, 1936; Pikes Peak Region, just east of Manitou, Johnston 2765 (CRB), June, 1920; Minnehaha, Pikes Peak, Johnston 2713 (CRB), June 2, 1920; Cog Road, Pikes Peak, Christ 168 (CRB), June 6, 1935. *Grand Co.*, Grand Lake, E. C. Smith (CRB), June 6, 1934. *Lake Co.*, Leadville, Eastwood 7143, 7144, 7154 (USN), June 19, 1819. *Larimer Co.*, Estes Park, elev. 7500 ft., E. C. Smith 446, 451 (CRB), June 11, 1934. *San Juan Co.*, Silverton, alt. 10,000 ft., Tweedy 268, 269 (USN, 268 doubtfully cited by Rydberg), July, 1895. *Teller Co.*, Divide, Christ 205, 206 (CRB), June 9, 1935; North Branch of Catamount Creek, elev. 9400 ft., E. R. Warren 9 (CRB), Sept. 2, 1926.

WYOMING: *Albany Co.*, Shrubs 3–8 ft. high, along stream, Nash's Fork, A. Nelson 7781, 7782 (CRB), July 28, 1900. Centennial, in bogs, Nelson 8823 (CRB), Aug. 7, 1902.

ZOOLOGY.—*Studies on trichinosis. X. The incidence of light infestations of dead trichinae in man.*[1] LEON JACOBS, National Institute of Health. (Communicated by W. H. WRIGHT.)

In a survey of the incidence of trichinae in man in the United States, begun at the National Institute of Health by Hall and Collins

[1] Received July 11, 1938.

(1937),[2] continued by Nolan & Bozicevich (1938),[3] and at present being carried on by the writer, two methods are used for the detection of trichina larvae in diaphragm muscle obtained at necropsy. These methods, which have been described in detail by the above-mentioned writers, are briefly, as follows:

The microscopic examination, in which one gram of muscle from around the tendinous portion of the diaphragm is pressed between two heavy glass plates in a metal press, and examined under the low power of the dissecting microscope (1.7× objective and 12.5× ocular). If live or dead trichinae are present in numbers large enough to be found by an examination of one gram, they will be revealed by this technique.

The digestion-Baermann technique, in which the major portion of the diaphragm is ground up and digested in artificial gastric juice. The residue, after sedimentation, is poured onto the screen of a Baermann funnel, and fluid from the bottom of the funnel is later drawn off and examined for trichinae. Live larvae, if present even in very small numbers, are detected by this method. Occasionally, also, calcified cysts will pass accidentally through the screen and will be found in the fluid at the bottom; the number so found, however, is not an indication of the intensity of infestation.

The two techniques supplement each other. The microscopic examination reveals live and dead cases when the infestation is of the order of at least one per gram, while the digestion-Baermann technique detects live larvae in any number. One-third of the positives are found by the microscopic examination alone, one-third by the digestion-Baermann technique alone, and one-third by both techniques. Only light infestations of dead trichinae of the order of less than one per gram can be consistently missed by both techniques. It was the purpose of the investigation recorded here to determine whether or not there exist in man infestations with dead trichinae of the order of less than one larva per gram, and if so, to what extent.

Material for this investigation was obtained from the diaphragms used in the routine studies. When large diaphragm samples were received, the major part, as usual, was digested, and one gram from around the tendinous portion was saved for the microscopic examination. An additional ten grams from regions around the tendinous portion were saved for the purpose of this second survey, and if the routine examinations showed the sample to be negative, these ten grams were examined for the presence of dead trichinae.

No attempt was made to sample the material systematically. The

[2] Hall, Maurice C. and Collins, Benjamin J. *Studies on trichinosis. I. The incidence of trichinosis as indicated by post-mortem examinations of 300 diaphragms.* Public Health Reports **52**(16): 468–490. 1937.

[3] Nolan, M. O. and Bozicevich, John. *Studies on trichinosis. V. The incidence of trichinosis as indicated by post-mortem examinations of 1000 diaphragms.* Public Health Reports **53**(17): 652–673. 1938.

sampling was left purely to chance, and depended upon the size of the diaphragm samples received and the amount of time available for work on the study. The first ten-gram sample came from diaphragm No. 1382, and the last from diaphragm No. 1874. Thus there was a random sampling of almost 500 diaphragms. Sometimes ten-gram samples from ten or more consecutive diaphragms were examined, with the double purpose of completing the survey as promptly as possible, and of ascertaining whether any positives were being missed when long series of negatives were found by the routine examinations. In this connection, it is interesting to note that only one case was found which was not detected, but should have been detected, by the routine digestion-Baermann technique. This case represented an infestation with only one live larva per 10 grams of material. Since that time, the digestion-Baermann technique has been changed slightly. Instead of sedimentation glasses into which the fluid from the Baermann funnel was drawn, and from the bottom of which, after an hour's standing, fluid was pipetted into Syracuse dishes, small funnels from the bottom of which the sediment can be drawn, have been introduced. The use of these funnels may lessen the possibility of missing a few live trichinae.

Of 100 diaphragms previously found negative in the routine examinations, the microscopic examination of an additional ten-gram sample revealed six cases positive for trichinae. One of these cases represented a live infestation, in which one uncalcified cyst containing a live larva was found in ten grams of muscle. Each of two other positives showed one uncalcified cyst containing a degenerated larva, and each of the remaining three cases showed one partially calcified cyst containing a degenerated or dead larva. In no case was an infestation of more than one cyst found in any of the ten-gram samples. Table 1 gives detailed results of the positive findings.

TABLE 1.—FINDINGS FOR POSITIVE CASES

Diaphragm	Larvae per 10 grams	Condition of Cyst	Condition of Larva
Number	Number		
1385	one	one pole calcified	degenerated
1437	one	uncalcified	alive
1590	one	partially calcified	degenerated
1617	one	uncalcified	degenerated
1694	one	uncalcified	degenerated
1709	one	polar calcification	dead

It appears significant to the writer that in none of the 100 examinations were positives revealed that had a greater probability than

one chance out of ten of being discovered in the routine examination of a one-gram sample. Theoretically, of course, there is a possibility of missing in the microscopic examination of a one-gram sample cases in which as many as nine cysts are present in ten grams. The writer intends to make another survey of 100 ten-gram samples from diaphragms revealing one cyst per gram in the routine microscopic examination, with the intent of determining the distribution of cysts in the muscle around the tendinous portion of the diaphragm. It is known that the larvae are more concentrated around the tendinous part of the diaphragm than elsewhere, but it seems possible, from the data presented above, that the larvae are more or less evenly distributed around the tendons.

In the regular trichinosis survey being conducted at the National Institute of Health, it has been found that approximately 17 per cent of the total number of diaphragms examined contained trichinae. According to the data presented here, probably 5 or 6 per cent of the remaining diaphragms had infestations that were missed in the routine examinations. This adds approximately four or five per cent to the total incidence figure.

ZOOLOGY.—*Notes on the "culture" of aquatic nematodes.*[1] B. G. CHITWOOD and M. B. CHITWOOD, Bureau of Plant Industry.

Most aquatic nematodes seem to require considerable aeration and it is not customary to keep such forms alive in the laboratory. To the writer's knowledge, no one has succeeded in keeping marine nematodes reproducing in the laboratory. Eventually, when their feeding habits are sufficiently understood, it seems possible that we may be able to culture aquatic nemas in test tubes. The first steps in this direction are reported in the following experiments wherein such forms have been raised in balanced aquaria.

Aquarium A.—A one quart aquarium bowl, maximum depth 3 inches, was half filled with stream water, to which a little sand, algae and a minute aquatic flowering plant were added. Culture begun October 1937. Water was added to compensate for evaporation. On April 26, 1938, male and egg-laying females of *Tylenchus filiformis* v. *abulbosus* n. var. were secured from the sediment. Gastrotrichs, rotifers and planarians also abounded in this culture. Water, pH 8.3.

Aquarium B.—A rectangular tank 28 by 12 by 10 inches was filled to a depth of 4 inches with sievings from stream, some algae and a small flowering plant (*Lemna* sp.). Kept covered to three-quarters of its length with a

[1] Received July 29, 1938.